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Review on the Role of Molecular Genetics in Animal Performance Improvement

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Abstract: This review was conducted to evaluate the role of molecular genetics in animal performance improvement. Molecular genetics is the field of biology and genetics that studies the structure and function of genes at a molecular level. There are opportunities for using molecular genetics to identify genes that are involved in variety of traits. Revolutionary opportunities for the modification of animal performance are being created by the development of new methods for embryo manipulation and the application of molecular biology.

Key words: Genetic Improvement · Molecular Marker · Animal

INTRODUCTION

Molecular genetics is the study of genetic makeup of individuals at the DNA level. It is the identification and mapping of genes and genetic polymorphism [1]. It employs the methods of genetics and molecular biology to elucidate molecular function and interactions among genes. It is so-called to differentiate it from other sub fields of genetics such as ecological genetics and population genetics. Along with determining the pattern of descendants, molecular genetics helps in understanding genetic mutations that can cause certain types of diseases. Through utilizing the methods of genetics and molecular biology, molecular genetics discovers the reasons why traits are carried on and how and why some may mutate (Wikipedia, the free encyclopedia). Livestock contribute directly to livelihoods worldwide, providing not only food, but also non-food products, draught power and financial security. However, land degradation, environmental pollution, global warming, the erosion of animal genetic resources, water shortages and emerging diseases are all expected to present challenges to the growing global livestock sector [2].

Conventional technologies and biotechnologies in livestock have contributed immensely to increasing productivity, particularly in developed countries and can help to alleviate poverty and hunger, reduce the threats of diseases and ensure environmental sustainability in developing countries. A wide range of biotechnologies are available and have already been used in developing countries in each of the three main sectors of animal sciences, which can be categorized as animal reproduction, genetics and breeding; animal nutrition and production; and animal health [2].

There are opportunities for using molecular genetics to identify genes that are involved in variety of traits. Armed with this information it would be possible to select improved livestock on the basis of their genetic makeup. If applied with care, the use of molecular information in selection programmes has the potential to increase productivity, enhance environmental adaptation and maintain genetic diversity [1]. Genetic improvement programs for livestock and crop species can be enhanced by the use of molecular genetic information in introgression, genotype building and recurrent selection programs [3]. The first task is to understand the genetic control of the trait of interest and then to identify the genes involved [1].

Revolutionary opportunities for the modification of animal performance are being created by the development of new methods for embryo manipulation and the application of molecular biology [4]. Now a day, because of interrelated problems coupled with climate change, both production and productivity of livestock is declined from time to time. Molecular genetics is now

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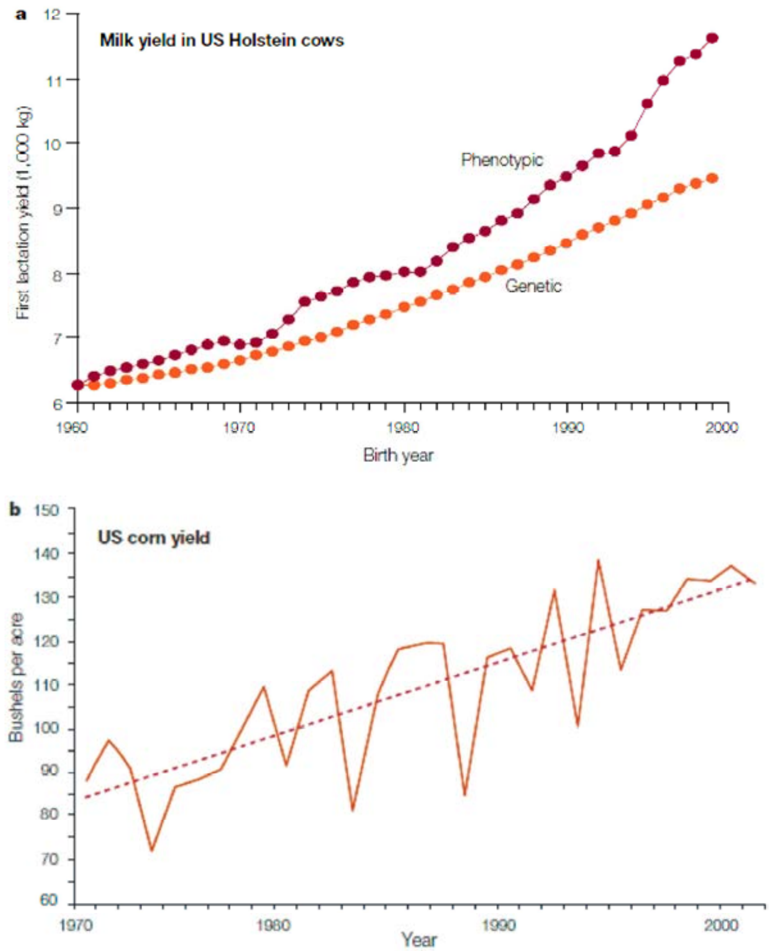

Fig. 1: Examples of genetic improvements in livestock and crops. a) Average milk production per lactation of US Holstein cows; b) For corn, yields have increased fourfold during the past 60 years

becoming a new emerging science to improve and upgrade the productivity of animals through genetics bases. This paper is then initiated to review on the role of molecular genetics in animal performance improvement.

Need for Molecular Genetics: The use of molecular genetics technologies potentially offer a way to select breeding animal at an early age (even embryos); to select for a wide range of traits and to enhance reliability in predicting the mature phenotype of the individual. The broad categories of existing gene based options include; molecular analysis of genetic diversity, animal identification and traceability, reproductive enhancement; transgenic Livestock; germ line Manipulation; gene based trait selection; animal health: diagnosis, protection and treatment and ruminant and non-ruminant nutrition and metabolism [1].

The Use of Molecular Genetics in the Improvement of Agricultural Populations: Substantial advances have been made in the genetic improvement of agriculturally animal and plant populations through artificial selection on quantitative traits. Most of this selection has been on the basis of observable phenotype, without knowledge of the genetic architecture of the selected characteristics [5]. However, continuing molecular genetic analysis of traits in animal and plant populations is leading to a better understanding of quantitative trait genetics. The genes and genetic markers that are being discovered can be used to enhance the genetic improvement of breeding stock through marker-assisted selection [3].

Genetic improvement through artificial selection has been an important contributor to the enormous advances in productivity that have been achieved over the past 50 years in plant and animal species (Fig. 1). Most of the traits that are selected are complex quantitative traits, which mean that they are controlled by several genes, along with environmental factors and that the underlying genes have quantitative effects on phenotype. So far, most selection has been on the basis of observable phenotype, which represents the collective effect of all genes and the environment [3]. Sophisticated testing and selection strategies have been developed and implemented for many species, with the aim of improving the genetic performance in a breed or line through recurrent selection or introgression. Another goal is to develop superior crossbreds or hybrids through the combination of several improved lines or breeds [3].

Genetic Improvement of Agricultural Species: Two important strategies for genetic improvement are recurrent selection and introgression programmes. The aim of selection programme, which is the main vehicle for genetic improvement in livestock, is to improve a breed or line as a source of superior germplasm for commercial production through within-breed or within-line selection. This involves recording the phenotypes of numerous individuals and the use of these phenotypes to estimate the 'breeding value' of selection candidates [3]. Selection commonly referring the natural selection; which is the process of differential survival and reproduction of individuals resulting in long-term changes in the characteristics of species. Natural selection is a three part process. First, there must exist differences among individuals in some trait. Second, the trait differences must lead to differences in survival and reproduction. Third, the trait differences must have a genetic basis. Natural selection results in long-term changes in the characteristics of the population [5]. Improvement of stock for commercial production often involves further product development through testing, breeding or crossing to generate crossbreds or hybrids. Introgression is another important genetic improvement strategy, in particular in plants. The aim of an introgression programme is to introduce a 'target' gene, which can be a single gene, a quantitative trait locus or a transgenic construct, from an otherwise low-productivity line or breed (donor) into a productive line that lacks that particular gene (recipient; R). Introgression starts by crossing the donor and recipient lines, followed by repeated backcrosses (BC) to the recipient line to recover the recipient-line genome. The target gene is maintained in the backcross generations through selection of donor gene carriers. Recovery of the recipient genome can be enhanced by the selection of backcross individuals that have a high value for the recipient trait phenotype [3].

Application of Molecular Genetic Technologies in Livestock Production: Methods have been developed to isolate animal genes and to characterize them. The first approach is to identify DNA sequence associated with economic trait loci. The second step is to incorporate the identified gene into three maps: (I) a physical map of chromosome (where DNA sequences are assigned to specific sites on specific chromosomes), (ii) a linkage map (where linkage of different genes and markers is assigned in the same chromosome), (iii) a genetic map (where inheritance of economic traits is corrected with the inheritance of genes and markers) [6].

Molecular Genetics Technologies: Application of molecular genetics in livestock production has been reviewed indicating that why molecular genetics is important in the presence of conventional livestock improvement programs. Those molecular genetics technologies which are applicable in livestock improvement are summarized in Fig. 2.

Applications of Molecular Markers: molecular markers can play an important role for livestock improvement through conventional breeding strategies. The various possible applications of molecular markers are short-range applications or immediate and long-range applications [1].

PCR Technology: The PCR is a method that efficiently increases the number of DNA molecules in a logarithmic and controlled fashion. PCR is a major scientific development and tag polymerase the enzyme essential to

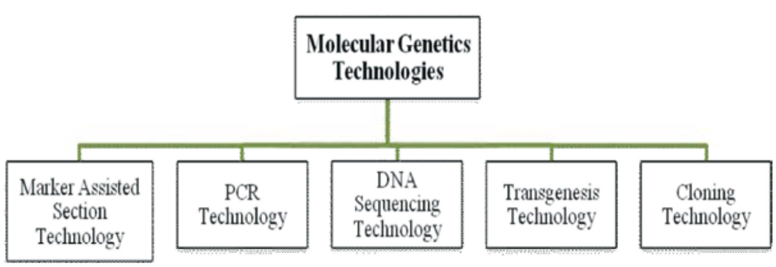

Fig. 2: Molecular genetics technologies

PCR's success. The chemistry involved in the PCR depends on the complimentarily (matching) of the nucleotide bases in the double stranded DNA helix. When a molecule of DNA is sufficiently heated, the hydrogen bands holding together the double helix are disrupted and the molecule separates or denature into single strand.

PCR can be used very effectively to modify DNA. Modification means that addition of restriction enzyme sites and generation of desired site directed mutations or deletions. PCR has a profound effect on all molecular studies including those in diagnostic area. The PCR has dramatically impacted on diagnosis of genetic and infectious disease. The PCR today plays a central role in genetic typing of organisms or individuals and molecular epidemiology [1].

DNA Sequencing Technology: DNA sequencing is the process of determining the exact order of the billions of chemical building blocks (called bases and abbreviated A, T, C and G) that make up the DNA. Genomics, the science of identifying the entire set of genes of living organisms is revolutionizing biological sciences and has become a driving force in mergers and divestments involving many of the world's largest corporations. DNA sequencing technology is now being used by public and private researchers to decipher the genetic blueprint of humans, plants, animals and micro-organisms [1].

Highest resolution of DNA variation can be obtained using sequence analysis. Sequence analysis provides the fundamental structure of gene systems. DNA sequencing is generally not practical to identify variation between animals for the whole genome, but is a vital tool in the analysis of gene structure and expression [1].

Cloning Technology: Cloning technology allows us to generate a population of genetically identical molecules, cells, plants or animals. Because cloning technology can be used to produce molecules, cells, plants and some animals, its applications are extraordinarily broad. Any legislative or regulatory action directed at "cloning" must take great care in defining the term precisely so that the intended activities and products are covered while others are not inadvertently captured [1].

Transgenesis: Gene transfer (or transgenesis) means the stable incorporation of a gene from another species in such a way that it functions in the receiving species and is passed on from one generation to the next. In mammalian species, the transfer is mostly done by direct injection of the foreign DNA into the nucleus at the early embryonic stage. Gene transfer has been achieved in all the major livestock species and since the first success in 1985, more than 50 different transgenic have been inserted into farm animals. Because so many separate steps are involved, the success rates are often low usually one or two percent. This imposes an enormous cost in the case of cattle; so most work has been done in mice, pigs and sheep [1].

Application of Molecular Markers in Improvement of Livestock: Usually a marker is considered as a constituent that determines the function of construction. Variations occurring at different levels i.e. at the morphological, chromosomal, biochemical or DNA level can serve as genetic markers. Thus genetic markers can be defined as any stable and inheritable variation that can be measured or detected by a suitable method and that can be used to detect the presence of a specific genotype or phenotype other than itself. The markers revealing variations at DNA level are referred to as 'molecular markers'. The molecular markers are classified into two broad categories, the hybridization-based markers and the PCR-based markers [6].

Properties of Molecular Markers: In genetic analysis, many types of markers like, morphological, chromosomal, biochemical and molecular markers are used. Morphological (e.g. pigmentation and other features) and chromosomal (e.g. structural and numerical variations) markers usually show low degree of polymorphism, therefore, they are not very useful. Biochemical markers have been tried out extensively but have not been found encouraging as they are sex linked, age dependent and influenced by the environment. The molecular markers capable of detecting the genetic variations at the DNA sequence level have removed the limitations. They possess unique genetic properties that make them more successful than the genetic markers. They are numerous, distributed on genome, follow typical mendalian inheritance and are multiallelic giving heterozygosis of more than 70 per cent and unaffected by environmental factors.

For genetic analysis, molecular markers offer several advantages; (i) the DNA samples can easily be isolated from blood, tissues e.g. sperms, hair follicle as well as archival preparations, (ii) the DNA samples can be stored for a longer time and readily be exchanged between the laboratories, (iii) the analysis of DNA can be carried out at an early age or even at the embryonic stage, irrespective of sex, (iv) once the DNA is transferred onto

Table 1: Molecular markers useful in conventional livestock breeding

No.	Application	Marker system
1.	Short-range/immediate Application	
	Parentage determination	DNA fragment polymorphic, microsatellite
	Genetic distance estimation	DNA fragment polymorphic, random amplified polymorphic DNA microsatellite
	Determination of zygosity / Freemartins	restricted fragment length polymorphism, polymorphic chain reaction - restricted fragment length polymorphism, microsatellite restricted fragment length polymorphism,
	Sex determination	polymorphic chain reaction - restricted fragment length polymorphism, microsatellite
	Identification of disease carrier	restricted fragment length polymorphism, cleaved amplified polymorphic sequence, microsatellite
2.	Long-range Application	
	Gene mapping	Type II markers e.g. variable number tandem repeat, minisatellite, microsatellite, random amplified polymorphic DNA
	Marker-assisted selection	Any marker having direct/indirect association with the performance traits/quantitative tri loci under question

a solid support e.g. filter membrane, it can be repeatedly hybridized with the different probes and heterogonous probes and in vitro synthesized oligonucleotide probes can also be used and (v) the PCR-based methods can be subjected to automation [6].

Application of Molecular Markers: Polymorphism observed at the DNA sequence level has been playing a major role in human genetics for gene mapping, pre- and post- natal diagnosis of genetic diseases and anthropological and molecular evolution studies. Similar approach for exploitation of DNA polymorphism as genetic markers in the field of animal genetics and breeding has opened vistas in livestock improvement programmes. Much interest has been generated in determining variability at the DNA sequence level of different livestock species and in their assessment whether these variations can be exploited efficiently in conventional as well as transgenic breeding programmes [6].

Molecular markers can play important role in livestock improvement through conventional breeding strategies. The various possible applications of molecular markers are: short-range (immediate) applications and the long-range applications (Table 1).

Transgenic Breeding Strategies: The current breeding strategies of livestock largely rely on the principle of selective breeding. In this method genetic improvement is brought about by increasing the frequency of advantageous alleles of many loci. The actual loci are rarely identified. In these methods genes cannot be moved from distant sources like different species or genera due to reproductive barrier. Trancegenesis, which is a recent development in molecular biology, removes these breeding barriers between different species or genera.

Trancegenesis has opened up many vistas in understanding behavior and expression of a gene. It has made possible to alter the gene structure and modify its function. There are many applications of Trancegenesis, but the most convincing one is the development of transgenic dairy animals for the production of pharmaceutical proteins in milk and animals with altered milk composition. The Trance genesis first starts with identification of the genes of interest. In this context, molecular markers can serve as reference point for mapping relevant genes. After successful production of transgenic animals, appropriate breeding methods could be followed for multiplication of transgenic herd. Molecular markers can also be used for identification of the animals carrying the transgenic. However, it is expected that molecular markers will serve as a potential tool to geneticists and breeders to evaluate the existing germoplasm and to manipulate it to create animals of desired traits as demanded by the society [6].

Transgenic Animals: Cell manipulation has been aimed at production of novel chemicals, pharmaceutical drugs or improvement in animal breed. The other purpose has been to study the structure and function of genes through molecular markers and genome mapping. Improvement in livestock has already given good results for increased milk production in catties, increased growth rate of livestock and fish, production of valuable proteins in large quantities in milk, urine and blood of livestock, wool production in transgenic sheep. Thus the transgenic animals can be used as bioreactors for large scale production of valuable recombinant chemicals such as hormones, interferons, proteins, etc. Thus, manufacturing of recombinant drugs through transgenic animals is called 'molecular farming' or 'molecular pharming' (Fig 3).

Desired foreign genes are transferred into animal cells/embryos via virus, microinjection, targeted gene transfer methods, etc.

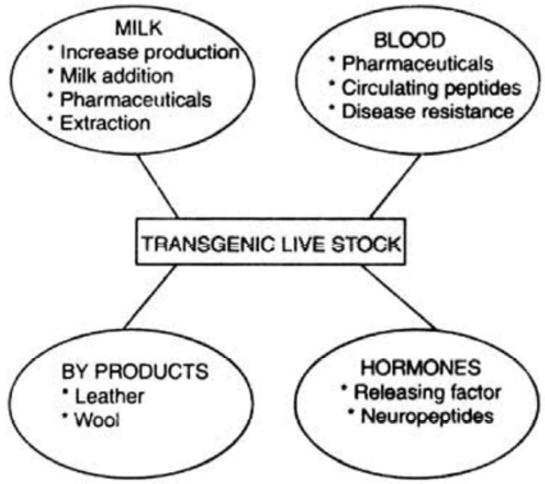

Fig. 3: Areas of investigation of domestic livestock via transgenic technology.

Manipulation of Reproduction in Animals: One of the challenges for genetic improvement is to increase reproduction rates. Several reproduction techniques are available. The commonest of these are artificial insemination (AI), embryo transfer and associated technologies. Measurement of progesterone in milk or blood which is a widely used technique for monitoring ovarian function and for pregnancy tests is also an important technology for managing the reproductive function of the animal [7].

Artificial Insemination: A male animal produces millions of sperms daily. Theoretically, it can inseminate females regularly and produce several offspring's. This excess capacity of male has been utilized through developing new technologies for artificial insemination which can be said as the first animal biotechnology (www.eplantscience.com). No other technology in agriculture except hybrid seed and fertilizer use has been so widely adopted at a global scale as AI. Progress in semen collection, dilution and cryopreservation now enables a single bull to be used simultaneously in several countries for up to100, 000 inseminations a year [7].

The most effective factor that has increased the productivity of cattle is the artificial insemination. However, the breeder must replace the nature through artificial insemination if he ensures about the ovulation of female in herd together at a time. In contrast, if a breeder waits for female to ovulate and then inseminate separately, the importance and economic significance of artificial insemination get reduced. Therefore, through artificial insemination technology increased number of females can be inseminated by a male. In dairy industry demand of females is more than the males. Secondly, females have more desirable characteristics. The livestock industry prefers animals of one sex. Therefore, through artificial insemination technology X and Y chromosomes can be detected and sex of progenies determined accordingly.

Embryo Transfer: The principal benefit of embryo transfer is the possibility to produce several progeny from the female, just as AI produces many offspring from one male animal. For example the average lifetime production of a cow can be increased from 4 to 25 calves. Embryo transfer is still not widely used despite its potential benefits. In developing countries this is mainly due to absence of the necessary facilities, infrastructure and technical difficulties and limited supply of embryo from super ovulated donors. However, even in developed countries commercial embryo transfer is still used only in specialized niches or for a small proportion of best cows in the best herds. This is mainly a cost consideration [7]. The embryo develops in foster mother (recipient) which simply acts as incubator and does not make any genetic contribution to the offspring. Secondly, ruminant female carries one pregnancy at a time as only one egg is produced and fertilized with male's sperm. Thus, there is a chance for increasing the number of egg production at a time and transfer of fertilized eggs i.e. embryo into uterus of less important foster mothers other than original female in farm animal.

Multiple Ovulations (Super Ovulation): The reproductive cycle of ruminant female is such that the ovarian follicle of a non-pregnant female matures and releases single egg at a time. Normally ovulation occurs as a result of circulation of gonadotropin hormone. But by increasing the concentration of hormone the number of egg production gets increased. However, this depends on health, nutrition, breed of animals and environment in which they live. Inhibit hormone which is produced by the fast growing egg impress the growth of other eggs released at the same time. Then, during multiple ovulations, the effect of inhibit will be suppressed by artificial application of gonadotropin and all ovarian follicles will be matured and ovulated. The females to be super ovulated are frequently injected with prostaglandin F2a (PGF2a) so that synchronized oestrous could develop in them. After 10 days of oestrous they are injected with hormone FSH up to 4 days followed by PGF2a treatment so that oestrous may be maintained. The FSH treatment induces super ovulation. The females are artificially inseminated. The eggs are fertilized. After fertilization embryos undergo developmental stages.

Multiple Ovulation with Embryo Transfer (MOET): After 6-8 days of fertilization (for sheep and goat) the embryos are recovered. During this stage embryos have come to morale and remain in female's oviduct. In cattle the embryos are recovered without surgery by inserting a catheter into oviduct. A saline solution is drained in oviduct and embryos flushed out through catheter into a storage bottle. The oviduct of sheep is small, therefore, it is exposed surgically and embryos are recovered by syringe adopting the same method for cattle. In storage bottle embryos settle down and solution decanted. Embryos with small volume of saline are poured into a Petri dish. Then they are examined under microscope. The identified embryos are transferred into synchronized recipient females (as artificial insemination), when they are in 6-8 days of cycle of embryo development, stored and transported or manipulated as desired. In general, one super ovulated female results into 5-6 progenies. In cattle 50-60 per cent of pregnancy can be achieved from embryo transfer method [6].

Advantages and Disadvantages of Ai and Embryo Transfer:

- The advantages of AI and ET are numerous and include:
  - An increased rate of genetic improvement;
  - Increased utilization of outstanding sires;
  - Increased utilization of outstanding dams with ET;
  - Better selection procedures;
  - Decreased risk of injury to humans, mares and stallions;
  - Decreased risk of disease;
  - Decreased risk of problems with overuse of a particular sire in an individual herd;
  - Extending the usefulness of particular individuals in space (interstate and international shipping) and in time (semen is viable long after the demise of a sire or during times when a sire is unavailable for breeding);
  - Increasing the number of mares (and bitches to some extent) bred per day or with a single ejaculate.
- However there are also disadvantages which includes.
  - Use of AI requires estrus detection in the bovine,
  - Ovulation prediction in the canine and equine and ovulation detection in the equine;
  - AI and ET are not allowed by all breeds;
  - ET requires synchronization of a recipient with the donor;
  - They require trained personnel and some special equipment (http://getdecisive.com/asp/default.asp)

***In vitro* Fertilization (IVF) Technology:** The term in vitro means in glass or in artificial conditions and IVF refers to the fact that fertilization of egg by sperm had occurred not in uterus but outside the uterus at artificially maintained optimum condition. In recent years the IVF technology has revolutionized the field of animal biotechnology because of production of more and more animals as compared to animal production through normal course. For example, an animal produces about 4-5 offspring's in her life through normal reproduction, whereas through IVF technology the same can produce 50-80 offspring's in her life. Therefore, the IVF technology holds a great promise because a large number of animals may be produced and gene pool of animal population can also be improved. In many countries, like India, more calves are obtained through succession of this technology.

The IVF technology is very useful. It involves the procedure: (i) eggs collection from ovaries of female donor, (ii) in vitro maturation of egg cultures kept in an incubator, (iii) fertilization of the eggs in test tubes by semen obtained from superior male and (iv) implantation of seven days old embryos in reproductive tract of other recipient female which acts as foster mother or surrogate mothers. These are used only to serve as animal incubator and to deliver offspring's after normal gestation period. The surrogate mothers do not contribute anything in terms of genetic makeup since the same comes from the egg of donor mother and semen from artificial insemination [6].

Application of Molecular Genetics for Disease Control: Disease resistance is a particularly important attribute of livestock in low-input livestock production systems in the developing world. Resistance to infectious diseases is often the critical determinant of the sustainability of such systems and improving resistance is perceived as a primary target for genetic improvement programmes [8].

The control of infectious disease of livestock is currently achieved by a number of mechanisms, including; chemical intervention, such as anthelmintics for nematode parasite control, acaricides for tick control and antibiotics for the control of many bacterial diseases, vaccination, sanitation and disinfection and culling, isolation and control of the movements of animal and/or animal products (9)

Disease control or management using host genetic resistance (i.e. exploiting genetic variation in disease resistance amongst hosts) is increasingly recognized as a key component of effective disease control, complementing or sometimes replacing existing strategies. Breeders and agricultural industries in the developed world have a variety of incentives to genetically improve host resistance to disease. Host resistance to disease is a low-cost and usually sustainable approach to disease control (10). Increasingly, other measures are failing as parasites evolve to resist chemical or vaccine control measures (11). Important examples include the evolution of resistance to anthelmintics by nematodes in all major sheep-producing countries, the evolution of resistance to antibiotics by bacteria and the evolution of resistance to vaccines by the virus causing Marek's disease. Also, legislative changes in many countries are increasingly restricting the use of antibiotics and other therapeutics in animal production systems. There are also examples of governments dictating breeding strategies to farmers, such as the programme to limit clinical expression of scrapie in sheep flocks in Western Europe, using selection for prion protein (PrP) genotypes associated with resistance to scrape. (It remains to be seen whether the PrP alleles said to be associated with resistance to scrape in sheep confer complete or partial resistance, or perhaps confer delayed onset of clinical signs of disease.) In the developing world, the majority of poor farmers face all the pressures experienced in more developed regions but either cannot afford or do not have access to therapeutic and vaccine control. In their systems, the genetically controlled resistance of the host is a critical component of effective disease control [8].

CONCLUSION

Genetic improvement programmes for livestock and crop species can be enhanced by the use of molecular genetic information in introgression, genotype building and recurrent selection programmes. The first task is to understand the genetic control of the trait of interest and then to identify the genes involved. From the review process, it is well understood that, the technologies addressed for the improvements of animal performance through molecular genetics approach have complications/ difficulties in application. Some of these technologies like AI are implemented in our country; but because of technical problems and absence of infrastructures, their achievements compared with the efficiency of the technologies is less.

Recommendations:

- ✓ For better achievements of the technologies, technical scale up for experts must be addressed before implementation,
- ✓ Awareness on the technologies to the implementing sites (Example; training for farmers) should be created. Hence, the failure of AI in our country is because of lack of technical skill of experts and awareness of users.
- ✓ As all technologies are implemented to upgrade the productivity of animals, there must be a detailed selection of animals which are fit for the technology.
- ✓ Before application of any technologies in our country, in particular, they must be evaluated for their applicability and importance in research centers, which have a great importance for further succession of the technology.

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